Original Article

Does Coronavirus Disease-19 Infection Affect Ovarian Reserve in Infertile Women? A Retrospective Study

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Abstract

Background: Previous studies have revealed menstrual changes following coronavirus disease-19 (COVID-19) disease. The potential impact of COVID-19 on female reproductive organs, ovary in particular, has not been investigated thoroughly. **Aims:** The aim of this study was to evaluate changes in serum levels of follicle-stimulating hormone (FSH), luteinising hormone (LH) and oestradiol (E2) following COVID-19 disease as a surrogate for the detection of ovarian vulnerability to SARS-CoV-2 infection. **Settings and Design:** In this retrospective study, hospital records of unexplained infertile women between 21 and 40 years old who have attended our institution's reproductive medicine unit for evaluation and/or treatment of infertility have been evaluated. **Materials and Methods:** Menstrual cycle day 2–5 serum follicle-stimulating hormone, luteinising hormone and E2 levels of 28 infertile women have been studied both before and after the COVID-19 disease to evaluate ovarian reserve before the ovulation induction treatment cycle. **Statistical Analysis Used:** The demographic characteristics and hormonal results of these 28 unexplained infertile women have been compared. The Shapiro–Wilk test has been used to evaluate the normal distribution of variables. Comparison of ovarian reserve markers which were established before and after COVID-19 infection has been performed using paired samples *t*-test. **Results:** All patients except one have shown mild COVID-19 symptoms and their infection courses have resulted in uneventful recovery. Serum FSH, LH and E2 levels of 24 (85%) and serum anti-Müllerian hormone (AMH) levels of 4 (15%) patients have been evaluated before and after COVID-19 disease is statistically similar. **Conclusion:** COVID-19 disease or inflammatory response of the infection itself does not seem to affect pituitary gonadotropins and ovarian hormones in infertile women based on menstrual cycle day 2–5 serum FSH, LH, E2 and AMH levels. Further studies including higher patient numbers are urgently needed to clarify the potential effects of COVID-19 disease on the gonadal function of women.

Keywords: *Coronavirus disease-19, female, infertility, ovarian reserve, severe acute respiratory syndrome-coronavirus-2*

Introduction

Novel coronavirus SARS-CoV-2 has spread globally since December 2019 causing a coronavirus disease-19 (COVID-19) pandemic resulting in millions

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of death due mainly to severe pneumonia. Vaccines have been developed and administered to decrease morbidity and mortality rates of the disease. Recent studies provided that male gonads might be potentially vulnerable to SARS-CoV-2 infection due to high levels of angiotensin-converting enzyme-2 (ACE2) receptors in male gonads which are target sites for SARS-CoV-2 virus.[1] Thickening of the basal layer of seminiferous tubules, decreased number of spermatozoa and Leydig cells, lymphocyte infiltration, and germ cell degeneration have been demonstrated in autopsies of men who died from COVID-19.[2] It has been discovered that the SARS-CoV-2 virus penetrates human cells by binding to ACE2 receptors present on the cell surface.[3] ACE2 pathway is also involved in ovarian follicle development and steroidogenesis and angiogenesis in response to gonadotropins.[4,5] Despite the effects of COVID-19 disease on the ovary has not been investigated thoroughly, the potential impact of the SARS-CoV-2 virus on embryonic implantation and the human placenta has previously been investigated.[6,7] In this study, we investigated the impact of SARS-CoV-2 virus infection on the ovarian reserve of infertile women.

Materials and Methods

In this retrospective study, hospital records of unexplained infertile women between 21 and 40 years old who have attended our institution's reproductive medicine unit for evaluation and/or treatment of infertility have been evaluated. The protocol for the research project has been approved by the Local Ethics Committee of the University of Health Sciences, Ankara City Hospital (Approval number: E2-21-65) where the study was undertaken and it conforms to the provisions of the Declaration of Helsinki (as revised in Tokyo 2004). The subjects who have been included in the study gave informed consent and patient anonymity has also been preserved. Power calculation could not be performed due to the lack of previous studies investigating the same topic. Seventy-seven infertile women have been detected who have been diagnosed with SARS-CoV-2 virus infection during the COVID-19 pandemic based on the hospital records of our institution's ovulation induction unit until October 2021. None of these women had been vaccinated before or after COVID-19 infection during the study period. Age, body mass index, infertility duration, gravidity, parity, and time passed after COVID-19 disease positivity of the patients have been recorded. Women with poor ovarian reserve, male factor infertility, tubal factor infertility, polycystic ovary syndrome, comorbid medical diseases, and currently on hormonal medication treatment have been excluded. We also excluded women with polycystic ovary syndrome due to already established hypothalamic hormonal imbalance for this endocrinological disease. Twenty-eight women have been detected whose cycle day 2–5 serum follicle-stimulating hormone, luteinising hormone and oestradiol (E2) levels have been studied both before and after the COVID-19 disease to evaluate ovarian reserve before the ovulation induction treatment cycle. Anti-Müllerian hormone (AMH) levels have also been studied in four of these 28 women.

Statistical analysis

Statistical analyses have been performed with SPSS version 22 by comparing the reproductive and hormonal results of these 28 infertile women. The Shapiro–Wilk test has been used to evaluate the normal distribution of variables. Comparison of ovarian reserve markers which were established before and after COVID-19 infection has been performed using paired samples *t*-test. *P* values below 0.05 have been accepted as statistically significant.

Results

In this retrospective study, we detected 28 unexplained infertile patients whose cycle day 2–5 serum follicle-stimulating hormone (FSH), luteinising hormone (LH), E2 and AMH levels were evaluated before and after infection of COVID-19 disease. All women but one had COVID-19 disease infection with mild symptoms and signs. None of them has been hospitalised due to this infection. These patients were seeking treatment for infertility and they were under investigation to establish an aetiology of their infertility and to plan further ovulation induction treatment. Among all patients, serum FSH, LH and E2 levels of 24 (85%) patients were evaluated and only 4 (15%) patients had serum AMH level evaluations before and after COVID-19 disease. Based on the Shapiro–Wilk test which was performed to evaluate distribution for normality of results, all parameters were within normal distribution. Demographic characteristics and demographic and clinical characteristics of the whole study group were shown in Table 1. All patients except one have shown mild COVID-19 symptoms and their infection courses have resulted in uneventful recovery. A comparison of serum FSH, LH, E2 and AMH levels of the patients based on the time period before and after COVID-19 disease infection has been presented in Table 2. None of the paired sample tests comparing serum hormone levels before and after COVID-19 infection were found to be statistically significant [Figure 1]. The comparisons were performed using paired samples *t*-test. The mean duration of evaluation for serum

Table 1: Clinical characteristics of the study group (*n*=28)

Parameter	*n*	Minimum	Maximum	Mean±SD
Age (years)	28	21	37	27.36±4.407
BMI (kg/m^2)	28	17.0	31.6	24.889±3.7358
Duration of infertility (years)	28	1	5	2.53±1.419
Gravidity (*n*)	28	0	3	0.50±0.839
Parity (*n*)	28	0	2	0.18±0.476
Abortion (*n*)	28	0	1	0.21±0.418
FSH level before COVID-19 infection	24	3	10	6.88±1.788
LH level before COVID-19 infection	24	1.7	27.2	6.396±5.4808
E2 level before COVID-19 infection	24	23	193	51.58±36.729
AMH level before COVID-19 infection	8	1.32	9.00	4.0163±3.18680
FSH level after COVID-19 infection	28	2.8	11.2	7.286±1.8954
LH level after COVID-19 infection	28	1.4	23.4	5.389±3.9561
E2 level after COVID-19 infection	28	11	110	46.11±20.375
AMH level after COVID-19 infection	12	0.01	9	2.92±2.446
Duration of serum hormone reevaluation following COVID-19 infection (months)	25	1	10	3.4±2.7

BMI=Body mass index, E2=Estradiol, FSH=Follicle stimulating hormone, LH=Luteinising hormone, AMH=Antimüllerian hormone, COVID-19=Coronavirus disease-2019, SD=Standard deviation, P=Progesterone, hCG=Human chorionic gonadotropin

Table 2: The comparison of basal hormone levels before and after coronavirus disease-2019 infection (*n*=28)

Parameter	*n*	Mean±SD			*P*
		Before COVID-19 infection	After COVID-19 infection	Difference	
FSH (IU/L)	24	6.88±1.78	7.25±1.99	−0.37±1.75	0.31*
LH (IU/L)	24	6.39±5.48	5.58±4.25	0.81±3.54	0.27*
E2 (pg/mL)	24	51.5±36.7	44±20	7.54±33.79	0.28*
AMH (ng/mL)	4	2.54±1.18	2.19±0.65	0.35±1.04	0.54*

**P* values were calculated by using paired samples *t*-test (The distribution of continuous variables is tested by using the Shapiro–Wilk test). E2=Oestradiol, FSH=Follicle-stimulating hormone, LH=Luteinising hormone, AMH=Antimüllerian hormone, COVİD-19=Coronavirus disease-2019, SD=Standard deviation

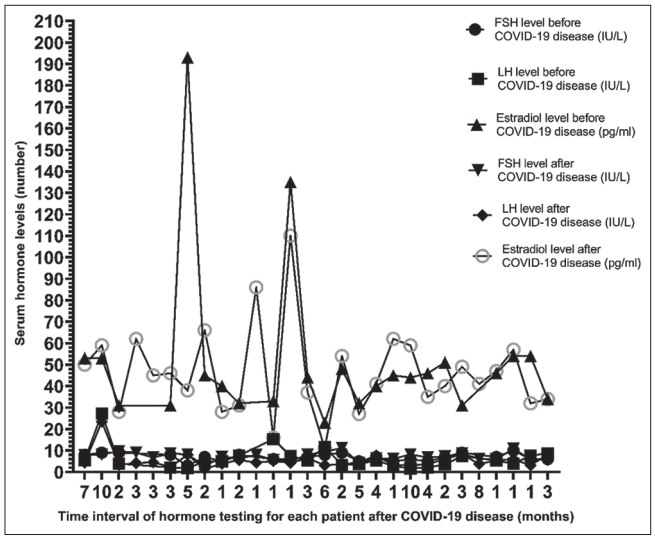

Figure 1: Serum levels of FSH, LH and oestradiol levels for each individual patient before and after COVID-19 disease based on time interval following the infection. FSH = Follicle-stimulating hormone, LH = Luteinising hormone

hormone levels following COVID-19 infection was 3.40 ± 2.784 months (minimum 1 month to maximum 10 months). Seventy-one per cent and 18% of the patients' serum hormone levels were evaluated earlier than 6 months and later than 6 months following COVID-19 infection, respectively.

Discussion

The SARS-CoV-2 virus infects the host cells through the ACE2 receptors with high affinity.[8] ACE2 receptors, which are widely expressed in the type II pneumocytes of the lungs, cardiovascular, gastrointestinal, urinary, neurological tissues and testes, are the target receptors for the SARS-CoV-2 viruses to replicate within these cells and release mature virions at last.[9]

ACE2 receptors are present in the male reproductive system. In females, gonadotropin-dependent expression of ACE2 has been reported in human ovaries.[10] Besides, ACE2 mRNA transcripts have also been detected in the ovaries of women.[11] ACE2 has been detected in stromal/granulosa cells and oocytes of immature rat ovaries just like bovine theca cells and granulosa cells.[12] In a recently performed study, Naigaonkar *et al.* have demonstrated that lower expression of "coronavirus-associated receptors and factors" in women

with polycystic ovarian syndrome (PCOS) which indicates that the risk of SARS-CoV-2 infection to the ovary may be lesser in these women.[13] This study delineates that susceptibility to the SARS-CoV-2 virus might be different among women with already established different intrinsic disease processes. Similarly, Colaco *et al*. have evaluated the coronavirus receptor expression in developing human embryos and they demonstrated that the embryonic cells have expressed genes involved in SARS-CoV-2 interactions and coronavirus infectivity.[6] Ashary *et al*. have studied whether the placental is permissive to SARS-CoV-2 virus infection or not and they have demonstrated that SARS-CoV-2 binding receptor "ACE2" and the S protein priming protease TMPRSS2 were co-expressed by a subset of syncytiotrophoblasts in the first trimester and extravillous trophoblasts in the second-trimester human placenta. They concluded that the placenta is a potential target for SARS-CoV-2 through these receptor viruses during pregnancy.[7] Ovarian effects of the SARS-CoV-2 virus are unknown and rarely studied before. Ovarian reserve markers and serum hormone levels might be surrogate predictors to investigate the potential effects of the SARS-CoV-2 virus on ovarian function. Despite the paucity of human studies about the presence/absence of ACE2 in the female gonads, the ovary might be a potential target for SARS-CoV-2 and SARS-CoV2 infection might have a deteriorating effect on ovarian function. Direct viral infection and ovarian cell destruction, excessive inflammatory response and dysfunction of pituitary gonadotropin and ovarian hormone secretion have been blamed for the abnormality of ovarian function caused by COVID-19 infection. Infection of the host cells by virus attack might not be the only way to impair organ function. An impairment of the cells and tissues can also be a result of systemic inflammatory responses induced by viral infection.[14] In this study, we have evaluated the change in cycle day 2–5 FSH, LH, E2 and AMH as a marker for the ovarian reserve of infertile women who have been diagnosed with COVID-19 infection. Based on previous studies, a virus attack might not be the only way to damage organ function. Respiratory failure, direct attack of ovarian tissue by the SARS-COV-2 virus or the immune response which is induced by the COVID-19 infection itself have been blamed for ovarian dysfunction.[15] By observation of increased serum LH and prolactin (PRL) levels, some studies have revealed that the central nervous system might be damaged by the SARS-CoV-2 virus which has been detected in the pituitary gland.[15,16] In these studies, despite other clinical or mental conditions like anxiety or depression that might affect serum FSH, LH, E2 and PRL hormone levels; direct viral effect on the pituitary gland has also been demonstrated. Ding *et al*. have compared women who have and who have not been diagnosed with COVID-19 infection and increased serum testosterone (T) levels due to increased LH levels' stimulation of thecal cells has been demonstrated which has been speculated to affect menstrual cycle parameters.[17] In our study, we compared serum hormone levels of the same patients before and after the COVID-19 infection and each patient has been assigned her control. Although it was statistically insignificant; serum LH levels and serum E2 levels were found to be lower following COVID-19 infection in our study. This decrease in serum E2 levels might be due to lowered serum LH levels and/or due to an effect on ovarian granulosa cells. In our study, it is hard to make comment on serum AMH level change following COVID-19 infection due to low patient number. Direct attack of the pituitary gland and/or ovarian follicles by the virus and exaggerated inflammatory response to viral infection might be potential causes for abnormal gonadotropin/gonadal hormone secretion and hypothalamic pituitary ovarian axis dysfunction. Currently, studies evaluating the long-term effects of COVID-19 disease on pituitary gonadotropin and ovarian hormone secretion of infertile women do not exist due to the relatively short time period following the surge of the global pandemic.

Limitations of this study should also be kept in mind. Despite an inverse relationship has been detected between serum FSH levels and COVID-19 infection, a causal relationship could not be proven due to low patient numbers, lack of knowledge for ovarian detection of SARS-CoV-2 virus infection and lack of evaluation for long-term change in serum hormone levels. Repeat serum hormone level checks were performed within a wide range of time period following COVID-19 infection. Evaluation of antral follicle count (AFC), as a measure of ovarian reserve, has not been performed during the study due to its nature of subjectivity. However, due to the paucity of studies evaluating serum gonadotropin hormone levels makes it hard to define the effects of COVID-19 infection on woman's ovarian function, the results of this study give an idea about a possible/probable effects of this pandemic infection on women's serum hormone levels. Another limitation of this study is that the severity of the COVID-19 disease among our patient group was mild and more severe COVID-19 infection could have resulted in more robust effect on ovarian function despite not proven with high-quality studies. Third, short-term and long-term effects of COVID-19 infection could have been different on ovarian function which is another topic worth investigating. Retrospective nature of the current study

precluded to stratify the patients based on the post-test time interval following COVID-19 infection. Ideally, ovarian reserve must be judged by evaluation of AFC and AMH both of which were not available for the present cohort. Future studies evaluating AFC and AMH which are the best ovarian reserve markers should be performed to verify our conclusions. Finally, the power of this study is low due to the low number of patients whose hormone levels have been evaluated before and after COVID-19 infection.

Conclusion

COVID-19 disease or inflammatory response of the infection itself does not seem to affect pituitary gonadotropin and ovarian hormone secretion in unexplained infertile women based on menstrual cycle day 2–5 serum FSH, LH, E2 and AMH levels. Further studies including higher patient numbers and investigating the ovulation induction cycle outcomes following COVID-19 infection to determine the association between infection/immunity/inflammation and ovarian function are needed in the future. Besides, the long-term effects of COVID-19 disease on pituitary gonadotropin and ovarian hormone secretion of infertile women should also be investigated with studies including a higher number of patients.

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Nil.

Conflicts of interest

There are no conflicts of interest.

Data availability

Our data are available for the journal if requested by the editor (s). Data can be shared as per requirement.

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